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Measurement of Blood-Brain Barrier Permeation in Rats during Exposure to 2450-MHz Microwaves

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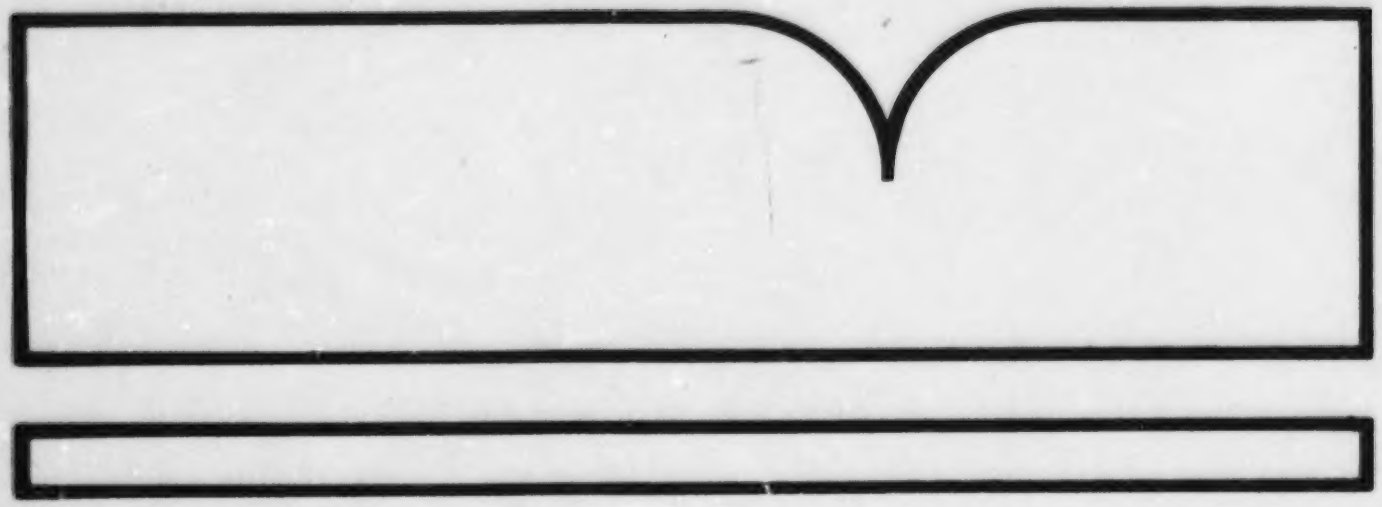

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16. ABSTRACT

Adult rats anesthesized with pentobarbital and injected intravenously with a mixture of [C] sucrose and [H] inulin were exposed for 30 min. to an environment at an ambient temperature of 22, 30, or 40 C, or were exposed at 22 C to 2450-MHz CW microwave radiation at power densities of 0, 10, 20, or 30 mW/cm. Following exposure, the brain was perfused and sectioned into eight regions, and the radioactivity in each region was counted. The data were analyzed by two methods. First, the data for each of the eight regions and for each of the two radioactive tracers were analyzed by regression analysis for a total of 16 analyses and Bonferroni's Inequality was applied to prevent false positive resuts from numerous analyses. By this conservative test, no statistically significant increase in permeation was found for either tracer in any brain region of rats exposed to microwaves. Second, a profile analysis was used to test for a general change in tracer uptake across all brain regions. Using this statistical method, a significant increase in permeation was found for sucrose but not for inulin. A correction factor was then derived from the warm-air temperature. This correction factor was applied to the data for the irradiated animals. After correcting the data for thermal effects of the microwave radiation, no significant increase in permeation was found.

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Measurement of Blood–Brain Barrier Permeation in Rats During Exposure to 2450-MHz Microwaves

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Adult rats anesthesized with pentobarbital and injected intravenously with a mixture of [^{14}C]sucrose and [^{3}H]inulin were exposed for 30 min to an environment at an ambient temperature of 22, 30, or 40 °C, or were exposed at 22 °C to 2450-MHz CW microwave radiation at power densities of 0, 10, 20, or 30 mW/cm^2. Following exposure, the brain was perfused and sectioned into eight regions, and the radioactivity in each region was counted. The data were analyzed by two methods. First, the data for each of the eight regions and for each of the two radioactive tracers were analyzed by regression analysis for a total of 16 analyses and Bonferroni's Inequality was applied to prevent false positive results from numerous analyses. By this conservative test, no statistically significant increase in permeation was found for either tracer in any brain region of rats exposed to microwaves. Second, a profile analysis was used to test for a general change in tracer uptake across all brain regions. Using this statistical method, a significant increase in permeation was found for sucrose but not for inulin. A correction factor was then derived from the warm-air experiments to correct for the increase in permeation of the brain associated with change in body temperature. This correction factor was applied to the data for the irradiated animals. After correcting the data for thermal effects of the microwave radiation, no significant increase in permeation was found.

Key words: blood–brain barrier, rats, 2450-MHz microwaves

INTRODUCTION

The blood–brain barrier is a functional concept that was first advanced by Ehrlich [1887] to explain selective transport of molecules into the intracellular compartment of the brain. In more recent times, the barrier has been identified as the endothelial cell layer that lines the brain capillaries [Brightman and Reese, 1969]. The endothelial lining differs from that of most other capillaries by the presence of tight junctions between cells and by low pinocytotic activity. These two features are apparently responsible for the restricted movement of ions, large molecules, and polar molecules from the capillary lumen into the brain. An opening of the tight junctions or an increase in pinocytotic

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activity would allow substances that normally remain in the blood to permeate the brain. Examples of physical agents that can increase the movement of substances into the brain include X rays and hyperthermia [Bradbury, 1979; Davson, 1976; Pardridge et al, 1975].

Scientists have studied the effects of microwave radiation on blood–brain barrier permeation, but the results as well as the interpretation of these results remain controversial [See reviews by Albert, 1979a and Justesen, 1980]. Frey et al [1975], Oscar and Hawkins [1977], and Albert [1977] have reported that microwaves can induce changes in the blood–brain barrier. Subsequent to these reports, Preston et al [1979] and Preston and Préfontaine [1980] found no change in permeation in laboratory animals exposed to microwave radiation. Merritt et al [1978] and Sutton and Carroll [1979] reported increased permeation due to microwave-induced hyperthermia, but no change when the brain was kept below 40 °C.

In our study, rats were injected with a mixture of two radiolabeled tracers and were exposed to microwaves or to an environment at one of several temperatures. Eight regions of the brain were then analyzed for uptake of the tracers. The data were tested for a microwave effect divorced from gross heating by comparing the amount of tracers in the brains of irradiated animals with the amount of tracers in the brains of animals exposed to an environment at temperatures that caused increases of core temperature comparable to those induced by microwaves. There are two features of our experimental protocol that differ from those of previous studies: (1) changes in barrier permeation were measured during far-field exposure and (2) permeation in irradiated rats was corrected for that due to elevation of whole-body temperature.

MATERIALS AND METHODS

Procedure

Ninety-day-old male CD albino rats that weighed approximately 350 g were purchased from the Charles River Animal Laboratories (Portage, MI) and were maintained on water and Purina rat chow ad libitum for at least a week prior to use.

The animals were first anesthetized by inhaled ether and then were injected intraperitoneally with sodium pentobarbital (50 mg/kg). Eight minutes after the injection both the left and right femoral veins were exposed surgically. Ten minutes after the pentobarbital injection, a mixture of [^{3}H]inulin and [^{14}C] sucrose [New England Nuclear] in equal parts of 0.9% saline and ethanol (33 μCi ^{3}H and 17 μCi ^{14}C/kg body weight in 300 μl/kg body weight) was injected into the right femoral vein. Five minutes after the isotopes were injected, a 0.4-ml blood sample was drawn from the left femoral vein. Rectal temperature was measured 10 min after the injection and again immediately after 30 min of exposure to microwave radiation or to an environment at a temperature of 22, 30, or 40 °C (Yellow Springs Instrument Model 46 TUC, probe 402). Ten minutes after exposure, a blood sample (0.4 ml) was drawn via cardiac puncture. The chest cavity was opened and the brain was perfused through the left ventricle with 0.9% saline (~100 ml). After 10 min of perfusion, the whole brain was removed and eight tissue samples were prepared by first removing the olfactory lobes and then sectioning the brain as described by Glowinski and Iversen [1966] into seven regions: cortex, hypothalamus, cerebellum, hippocampus, striatum, medulla, and midbrain. The eight samples from each brain were weighed, solubilized in 2 ml of NCS (Amersham Searle) at 37 °C for 48 h, and then placed in a liquid scintillation counter for measurement of ^{14}C and ^{3}H activity after 0.5 ml of 3 N HCl and 10 ml of scintillation fluid were added (ACS, Amersham Searle).

The blood samples were allowed to clot and 50-λ serum samples were added to 1 ml of NCS. After incubation at 37 °C for 48 h, the samples were acidified with 0.25 ml of 3 N HCl and 10 ml of scintillation fluid were added to each sample. Then the ^{3}H and ^{14}C activity in the serum samples was measured in a liquid scintillation counter.

Microwave Exposure and Environmental Temperature

Anesthesized rats were exposed individually from above to 2450-MHz CW radiation (long axis of the body parallel to the electric field) in the far-field of an anechoic exposure facility at 22 °C [Elder and Ali, 1975]. The power densities were 0, 10, 20 or 30 mW/cm^2 and the whole-body-averaged specific absorption rates (SAR) were approximately 0, 2, 4, or 6 W/kg. The SAR values are estimated from data on elevations of core temperature during exposure and are in agreement with data based on calorimetric measures [Gage et al, 1979]. A warm-air oven (Sargent Analytical Model S64080) that contained a small fan to circulate the air was used to expose rats to an environment at a temperature of 22, 30, or 40 °C. Eight animals were used at each power density and at each environmental temperature.

Urea Injection

Another group of animals (N = 9) received an injection of hypertonic urea which is known to disrupt the blood–brain barrier [Rapoport et al, 1972]. Only [^{3}H]inulin was used as a tracer in animals given urea. The schedule for this study was identical to that of the above experiments, and the procedures were the same through the 5-min drawing of the blood sample. Ten minutes after the inulin was injected, 0.3 ml of 8 M urea, pH 7.4, was injected (30-gauge needle) as a bolus into a common carotid artery, which had been surgically exposed. The animal was maintained at an ambient temperature of 22 °C during the 30-min treatment period, then at room temperature for 10 min until the second blood sample was drawn and the perfusion initiated. After perfusion, the brain was divided midsagittally, and each hemisphere was sectioned into the eight regions. By this procedure, the uptake of the radioisotope into the regions in the hemisphere affected by the urea injection could be compared with uptake in the noninjected or control hemisphere.

Analysis of Data

The permeation of the tracers is expressed as a blood-to-brain transfer constant, K_i. The transfer constant is the amount of tracer that crosses the capillaries (blood to brain) per unit time; it is an estimate of the product of brain-vascular area and permeability [Blasburg, 1979].

The transfer constants were calculated from the equation

$$K_i = T_b / \int_0^t Ca\ dt,$$

where T_b is the amount of tracer in the brain region (DPM/g wet weight) at t = 50 min, and $\int_0^t Ca\ dt$ is the integral of the serum concentration, Ca (DPM/ml), of the tracer from the time of injection until t = 50 min. This integral was determined experimentally by plotting the change in tracer concentration with time and calculating the area under the curve. An example of data from an animal injected with [^{3}H]inulin is shown in Figure 1 (broken line). Empirically, a good estimate (~97%) of the area under this curve was found to be the area under the curve determined by only the 5- and 50-min serum samples (solid line, Fig. 1). This procedure for estimating the integral also overcame the technical problem of taking blood samples during the 30-min exposures.

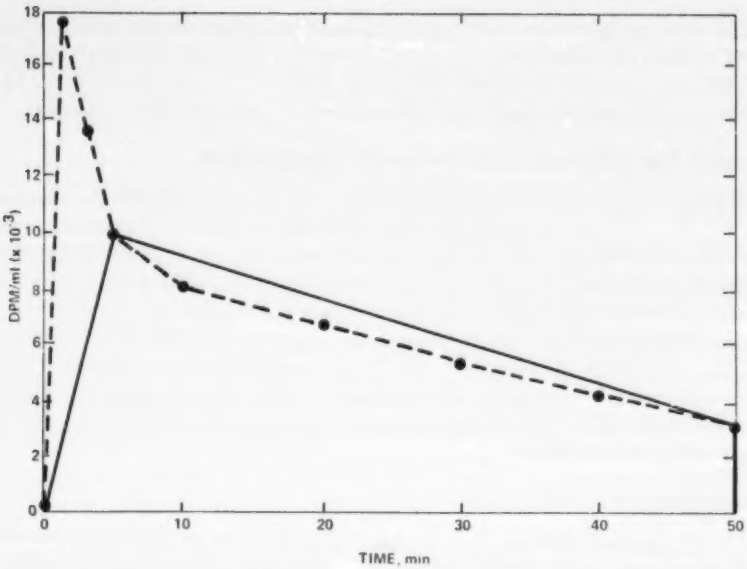

Fig. 1. Comparison of the estimated and true serum level of tracers. The broken line shows the true level of tracer (inulin) in the serum for the 50-min period, while the solid line shows the estimate of tracer determined by only the 5- and 50-min serum samples. The area under the broken line is within 3% of that under the solid line.

Statistical Procedures

The data from the environmental temperature and microwave experiments were analyzed by two methods. First, a series of regression analyses were performed to test for changes in individual brain regions. Second, a profile analysis was used to determine overall effects.

Individual regression analyses. The data for each of the eight regions and for each of the two tracers were analyzed by regression analysis for a total of 16 analyses. To prevent false positive results from numerous analyses, Bonferroni's Inequality was applied [Wallenstein et al, 1980; Lee et al, 1980]. With this procedure, the $P \leq .1$ normally used for significance at the 5% level in a one-sided test was divided by 16, the total number of independent analyses. Therefore, we used $P \leq .006$ as a significance level for this particular statistical test. Similarly, a significance level of $P = .1/8 = .013$ was derived for the urea experiment in which only one tracer was used (these data were analyzed by paired t tests).

Profile analysis. This analysis [Morrison, 1967] was used to test for a general change in tracer uptake across all brain regions. A preliminary test (Hotelling-Lawley trace statistic) required to validate this type of analysis was performed [Morrison, 1967].

RESULTS

Significant increases were found in [^{3}H]inulin permeation into four brain regions (cortex, striatum, hypothalamus, and hippocampus) from rats given hypertonic urea (Table 1). These results indicate that our technique could detect an increase in blood–brain barrier permeation that resulted from osmotic shock.

The change in rectal temperature of rats exposed to microwaves is shown in Table 2. At 0 mW/cm^2, the rectal temperature fell 0.6 °C. At 10, 20, and 30 mW/cm^2, increases

TABLE 1. Effect of Hypertonic Urea on Inulin Permeation

Brain region	K_i ($s^{-1} \times 10^6$)[a]			
	Control	Treated	ΔK_i	P[b]
Cortex	2.15 ± 0.29	9.76 ± 1.70	7.61 ± 1.46	.001
Cerebellum	2.43 ± 0.36	2.90 ± 0.54	0.47 ± 0.19	.036
Medulla	2.17 ± 0.35	2.37 ± 0.35	0.20 ± 0.31	.550
Striatum	1.16 ± 0.18	9.13 ± 2.07	7.97 ± 1.99	.004
Hypothalamus	2.52 ± 0.44	14.51 ± 3.49	11.99 ± 3.18	.006
Hippocampus	2.32 ± 0.29	10.40 ± 2.40	8.08 ± 2.13	.005
Midbrain	2.55 ± 0.46	8.22 ± 2.34	5.67 ± 2.12	.033
Olf. lobes	5.25 ± 1.02	10.04 ± 3.29	4.77 ± 2.75	.120

[a]Values are the mean transfer constants ± SEM (N = 9).
[b]The injected side (treated) of the brain was compared with the noninjected side (control) using the paired t test. Based on Bonferroni's Inequality, a $P \leq .013$ (0.1/8) is needed for significance at the 5% level.

TABLE 2. The Effect of Microwave Exposure and Environmental Temperature on Rectal Temperature[a]

Treatment	Rectal temperature (°C) ± SEM		
	Initial	Final	ΔT
Power density[b] (mW/cm^2)			
0	35.4 ± 0.1	34.8 ± 0.2	−0.6 ± 0.3
10	35.7 ± 0.3	35.9 ± 0.1	0.2 ± 0.2
20	35.9 ± 0.2	37.1 ± 0.2	1.2 ± 0.1
30	35.4 ± 0.2	37.4 ± 0.2	2.0 ± 0.1
Environmental temperature (°C)			
22	36.2 ± 0.1	34.6 ± 0.2	−1.6 ± 0.1
30	35.9 ± 0.2	36.1 ± 0.2	0.2 ± 0.1
40	35.7 ± 0.2	38.4 ± 0.2	2.7 ± 0.1

[a]Rectal temperature measurements were made in anesthesized rats before and after the 30-min treatment (N = 8 for each dose group).
[b]The temperature in the microwave chamber was 22 °C.

of 0.2, 1.2, and 2.0 °C, were measured, respectively. Other animals were kept for 30 min at environmental temperatures of 22, 30, or 40 °C, which produced changes in rectal temperature that spanned the range caused by microwave irradiation. For example, the temperature decreased 1.6 °C at 22 °C, and increased 0.2 °C at 30 °C and 2.7 °C at 40 °C (see Table 2). In both experiments, the change in body temperature was found to be directly proportional to environmental temperature and to power density. This result was expected because the anesthetic, pentobarbital, compromises thermoregulation in the rat such that its body temperature reflects thermal influences such as the ambient temperature and microwave radiation. We attribute the difference in rectal temperature at 22 °C in the warm-air oven ($\Delta T = -1.6$ °C) and in the microwave chamber ($\Delta T = -0.6$ °C at 0 mW/cm^2) to a combination of higher air flow in the warm-air oven and to the higher initial temperature of the rats kept at an environmental temperature of 22 °C (see Table 2).

The permeation of inulin and sucrose into the eight brain regions of rats exposed to microwaves is shown in Table 3. Although some P values obtained by individual regression analysis are less than 0.1, values often associated with significance, none was significant ($P < .006$) by Bonferroni's method (see Statistical Procedures).

TABLE 3. Permeation of Inulin and Sucrose in the Brain During Exposure to Microwave Radiation

Brain region	K_i, inulin ($s^{-1} \times 10^6$)[a] Power density (mW/cm^2) (0)	(10)	(20)	(30)	P*	K_i, sucrose ($s^{-1} \times 10^6$)[a] Power density (mW/cm^2) (0)	(10)	(20)	(30)	P*
Cortex	1.03 ± 0.10	1.17 ± 0.09	1.26 ± 0.09	1.23 ± 0.16	.14	2.72 ± 0.13	2.90 ± 0.21	2.96 ± 0.19	3.20 ± 0.11	.05
Cerebellum	1.10 ± 0.13	1.25 ± 0.11	1.41 ± 0.11	1.44 ± 0.16	.04	3.00 ± 0.14	3.15 ± 0.22	3.28 ± 0.19	3.56 ± 0.14	.02
Medulla	0.94 ± 0.13	1.01 ± 0.10	1.23 ± 0.09	1.25 ± 0.13	.03	2.59 ± 0.13	2.79 ± 0.16	2.83 ± 0.11	3.14 ± 0.13	.01
Striatum	0.50 ± 0.14	0.53 ± 0.12	0.68 ± 0.09	0.43 ± 0.09	.79	1.77 ± 0.08	1.85 ± 0.17	1.95 ± 0.08	2.05 ± 0.08	.06
Hypothalamus	0.92 ± 0.16	1.23 ± 0.16	1.05 ± 0.12	1.22 ± 0.16	.30	2.72 ± 0.19	2.87 ± 0.18	2.83 ± 0.18	3.19 ± 0.12	.07
Hippocampus	0.83 ± 0.12	0.82 ± 0.06	0.96 ± 0.15	0.96 ± 0.15	.32	2.23 ± 0.13	2.18 ± 0.08	2.18 ± 0.11	2.39 ± 0.11	.25
Midbrain	0.89 ± 0.13	1.01 ± 0.10	1.08 ± 0.09	1.20 ± 0.16	.04	2.58 ± 0.13	2.52 ± 0.14	2.63 ± 0.11	2.96 ± 0.18	.06
Olf. lobes	1.69 ± 0.21	1.58 ± 0.20	1.58 ± 0.10	1.73 ± 0.18	.90	3.88 ± 0.16	3.75 ± 0.30	3.26 ± 0.19	3.86 ± 0.24	.57

[a]Values are the mean transfer constants ± SEM of eight animals for each of the four exposure conditions.
*P values were derived by regression analysis.

Table 4 shows the transfer constants for inulin and sucrose in rats exposed at ambient temperatures of 22, 30, or 40 °C. As shown by individual regression analyses, only sucrose permeation of the hypothalamus increased significantly, but the absolute value of the transfer constants was greater in animals exposed at the highest temperature. These results indicate that permeation might have been influenced by core temperature. Therefore, we derived a correction factor to adjust the data for each brain region from microwave-exposed animals for permeation from whole-body heating caused by absorption of microwave energy.

From the warm-air exposure experiments, the first term, S_i, in the correction factor was obtained from plots of K_i versus the change in rectal temperature. The slope, S_i, of these lines varied with each tracer and brain region as shown in Table 5. From the microwave data in Table 2, the average rise of rectal temperature was found to be 0.09 °C per mW/cm^2. From these results, the change in permeation due to whole-body heating by microwave energy can be expressed as $(S_i)(.09 \times$ power density). This correction is based on the assumptions that microwave irradiation and the warm-air exposures cause equal changes in blood–brain barrier permeation per °C rise in core temperature and that this change is linearly related to core temperature over the range studied.

TABLE 4. Permeation of Inulin and Sucrose in the Brain During Exposure to Various Environmental Temperatures

	K_i, inulin ($s^{-1} \times 10^6$)[a]				K_i, sucrose ($s^{-1} \times 10^6$)[a]			
	Temperature (°C)				Temperature (°C)			
Brain region	(22)	(30)	(40)	P*	(22)	(30)	(40)	P*
Cortex	1.35 ± 0.10	1.30 ± 0.09	1.47 ± 0.09	.48	2.86 ± 0.11	2.85 ± 0.09	3.01 ± 0.05	.20
Cerebellum	1.41 ± 0.09	1.38 ± 0.09	1.51 ± 0.07	.48	3.22 ± 0.13	3.20 ± 0.11	3.50 ± 0.09	.09
Medulla	1.20 ± 0.06	1.25 ± 0.09	1.27 ± 0.06	.74	2.79 ± 0.09	2.81 ± 0.09	3.05 ± 0.08	.03
Striatum	0.88 ± 0.09	0.91 ± 0.10	1.00 ± 0.09	.35	2.03 ± 0.10	2.08 ± 0.12	2.17 ± 0.07	.19
Hypothalamus	1.05 ± 0.08	1.16 ± 0.12	1.40 ± 0.10	.03	2.82 ± 0.10	2.77 ± 0.12	3.21 ± 0.10	**
Hippocampus	0.96 ± 0.09	1.07 ± 0.09	1.13 ± 0.09	.34	2.31 ± 0.11	2.33 ± 0.08	2.47 ± 0.08	.15
Midbrain	1.18 ± 0.10	1.22 ± 0.07	1.33 ± 0.07	.24	2.75 ± 0.09	2.85 ± 0.11	3.01 ± 0.08	.06
Olf. lobes	1.93 ± 0.12	1.81 ± 0.12	1.90 ± 0.12	.83	3.52 ± 0.12	3.54 ± 0.16	3.82 ± 0.12	.28

[a]Values are the mean transfer constants ± SEM of eight animals for each of the four exposure conditions.
*P values were derived by regression analysis.
**P = .006

TABLE 5. Values of S_i *

Brain region	S_i, inulin[a] (s^{-1} °C$^{-1} \times 10^6$)	S_i, sucrose[a] (s^{-1} °C$^{-1} \times 10^6$)
Cortex	0.021 ± 0.030	0.036 ± 0.028
Cerebellum	0.019 ± 0.027	0.058 ± 0.034
Medulla	0.008 ± 0.022	0.058 ± 0.025
Striatum	0.023 ± 0.026	0.038 ± 0.028
Hypothalamus	0.072 ± 0.032	0.101 ± 0.035
Hippocampus	0.027 ± 0.027	0.042 ± 0.028
Midbrain	0.030 ± 0.025	0.057 ± 0.029
Olf. lobes	−0.008 ± 0.039	0.050 ± 0.046

*The slopes, S_i, were obtained from plots of K_i versus the change in rectal temperature caused by the warm-air exposures.
[a]Values are the means ± the standard error of estimate of the slope.

TABLE 6. Uptake of Inulin and Sucrose Corrected for Permeation Due to Increase in Core Temperature

Brain region	K_i, inulin ($s^{-1} \times 10^6$)[a]					K_i, sucrose ($s^{-1} \times 10^6$)[a]				
	Power density (mW/cm^2)					Power density (mW/cm^2)				
	(0)	(10)	(20)	(30)	P*	(0)	(10)	(20)	(30)	P*
Cortex	1.03 ± 0.10	1.16 ± 0.09	1.22 ± 0.09	1.17 ± 0.16	.27	2.72 ± 0.13	2.86 ± 0.21	2.90 ± 0.19	3.10 ± 0.11	.11
Cerebellum	1.10 ± 0.13	1.24 ± 0.11	1.38 ± 0.13	1.38 ± 0.16	.08	2.98 ± 0.14	3.09 ± 0.22	3.17 ± 0.19	3.41 ± 0.14	.09
Medulla	0.94 ± 0.13	1.01 ± 0.10	1.20 ± 0.09	1.23 ± 0.13	.04	2.57 ± 0.13	2.74 ± 0.16	2.72 ± 0.11	2.97 ± 0.02	.06
Striatum	0.50 ± 0.14	0.50 ± 0.12	0.64 ± 0.09	0.37 ± 0.09	.51	1.77 ± 0.08	1.82 ± 0.17	1.88 ± 0.08	1.95 ± 0.08	.22
Hypothalamus	0.92 ± 0.16	1.17 ± 0.16	0.93 ± 0.12	1.03 ± 0.16	.90	2.72 ± 0.19	2.78 ± 0.18	2.64 ± 0.18	2.92 ± 0.12	.48
Hippocampus	0.83 ± 0.12	0.80 ± 0.06	0.89 ± 0.15	0.88 ± 0.15	.59	2.23 ± 0.13	2.13 ± 0.08	2.12 ± 0.11	2.27 ± 0.11	.69
Midbrain	0.88 ± 0.13	0.98 ± 0.10	1.02 ± 0.09	1.12 ± 0.16	.12	2.58 ± 0.13	2.47 ± 0.14	2.52 ± 0.11	2.81 ± 0.18	.23
Olf. lobes	1.68 ± 0.21	1.61 ± 0.20	1.61 ± 0.10	1.75 ± 0.15	.83	3.88 ± 0.16	3.70 ± 0.29	3.17 ± 0.19	3.72 ± 0.24	.62

[a]Values are the mean transfer constants ± SEM of eight animals for each of the four exposure conditions.
*P values were derived by regression analysis.

The corrected transfer constants are shown in Table 6 along with P values from individual regression analyses. Again none is significant after applying the Bonferroni Inequality. A comparison of Tables 3 and 6 reveals that the correction reduced the transfer constants by a small amount, ie, ≤ 16% at the highest power density, and increased 14 of the 16 P values.

The results of the second statistical analysis (multivariate analyses) are shown in Table 7. First, Hotelling-Lawley trace statistics [Morrison, 1967] were evaluated to determine if profile analysis was appropriate. These statistics tested for the interaction between the 16 dependent variables and the linear effect of the independent variables. No significant interaction would indicate that profile analysis was appropriate and that the transfer constants for each of the eight brain regions behaved in a similar manner with respect to ambient temperature or microwave irradiation. As shown in Table 7, the Hotelling-Lawley tests were not significant. The statistical power of this test was investigated. In particular, it was questioned if this test could detect a 50% change due to microwave exposure in one brain region and for one tracer type, while the other 15 combinations of tracer types and brain regions were unaffected. Simulated normal data having the same average standard deviation (0.28) as observed in this study were generated and the significance was evaluated by this test. The P value was .0013 which is far below the 5% level. This suggests that there was sufficient power to detect such a pattern and somewhat weaker patterns if they had existed.

Although profile analysis was done on both the linear effect of the independent variable and a general change in means (except for environmental temperature where only the linear effect was tested), only the linear effect is reported in Table 7 because the P values were smaller by this method. The interaction between sucrose and inulin transfer constant profiles and environmental temperature was significant (P = .033). This interaction was borderline significant (P = .075) in the case of uncorrected transfer

TABLE 7. Statistical Results of Multivariate Analysis

Dependent variable Independent variable	Uncorrected transfer constant Environmental temperature	Uncorrected transfer constant Microwave power density	Corrected transfer constant[a] Microwave power density
A. Hotelling-Lawley test (interaction between 16 dependent variables[b] and independent variable)			
	$F_{(16,4)} = 1.21$, P = .473	$F_{(16,11)} = 1.29$, P = .340	$F_{(16,1)} = 1.16$, P = .410
B. Profile analysis			
1. Interaction between inulin and sucrose transfer constant profiles and independent variable	$F_{(2,18)} = 4.13$, P = .033	$F_{(2,25)} = 2.88$, P = .075	$F_{(2,25)} = 1.54$, P = .234
2. Linear effect of independent variable on inulin or sucrose transfer constant profiles			
(a) Inulin	$F_{(1,19)} = 0.38$, P = .543	$F_{(1,26)} = 2.80$, P = .107	$F_{(1,26)} = 1.56$, P = .223
(b) Sucrose	$F_{(1,19)} = 8.17$, P = .010	$F_{(1,26)} = 5.57$, P = .026	$F_{(1,26)} = 2.94$, P = .098

[a]Corrected for change in permeation due to change in rectal temperature caused by the microwave exposure (See Table 6).

[b]Eight brain regions, two radioactive tracers.

constants and microwave power density. Because the profiles were different, the linear effect of the independent variables on inulin and sucrose-transfer-constant profiles was tested separately. The inulin profile was insignificant in each case. The sucrose profile changed significantly both with change in environmental temperature ($P = .010$) and with microwave power ($P = .026$). However, there was no significant change in permeation due to microwave exposure after correcting for temperature. The P value of .098 for sucrose is not judged to be significant, because it would need to be smaller than .05 after applying a Bonferroni correction factor of two due to the two profiles being tested.

DISCUSSION

To determine whether a change in brain permeation could be detected by our technique, a hypertonic solution of urea was injected into one group of animals. Hypertonic urea disrupts the blood–brain barrier by osmotically shrinking the capillary endothelial cells, which opens the tight junctions between the cells [Rapoport et al, 1972]. Since the disruption is due to an osmotic effect, the urea is primarily effective on its first pass through the brain before it becomes diluted. Furthermore, while some of the hypertonic urea injected into one common carotid artery can cross to the opposite side of the brain, the osmotic shock predominantly affects the brain hemisphere on the injected side. Therefore, each animal served as its own control with one hemisphere of the brain affected by the hypertonic solution and the other hemisphere unaffected. The osmotic effect of the hypertonic urea should be largest in the cortex, striatum, hypothalamus, and hippocampus, which are supplied almost exclusively by the common carotid artery. Significant increases in [^{3}H]inulin permeation into these four brain regions were found, which indicate that our technique could detect an increase in blood–brain barrier permeation that resulted from osmotic shock.

In our technique, a 10-min perfusion of saline was used to clear the brain vasculature of blood so that brain counts represented extravascular tracer only. During perfusion, tracer back-diffusion from the brain into the saline-filled vasculature probably occurred which diminished the level of extravascular radioactivity and therefore could cause the K_i values to be low. The other method for measuring K_i is to estimate the residual blood in the brain after decapitation [Preston and Préfontaine, 1980]. However, this technique has a potentially large error due to the high level of tracer in the blood compared with the amount in the extravascular space. (Note that K_i and PA (permeability area product), the terminology used by Preston and Préfontaine [1980], are equivalent [Blasburg, 1979].)

As stated earlier, the data were analyzed both by a series of individual regression analyses and by profile analyses. By using a series of individual analyses, the response of each brain region to the microwave radiation can be determined. With this type of analysis, there is the possibility of false positives resulting from the repeated analyses. To prevent the false positive, Bonferroni's Inequality was used, yet when applying this inequality one tends to arrive at a very conservative significance level ($P \leq .006$ in our case). Nevertheless, the P values determined by individual analyses of the brain regions and tracers can give indications of effects in specific regions. When the data were analyzed by 16 independent regression analyses and Bonferroni's Inequality invoked, no significantly increased permeation was found by either tracer in any brain region. Yet 9 of 16 P values obtained were less than the .1 usually associated with significance.

The profile analysis, on the other hand, tests for general changes but does not indicate specifically which brain regions are affected or unaffected. This analysis revealed significant increases in sucrose permeation both in the irradiated animals and in the animals subjected to warm-air environments (see Table 7). No significant change in permeation by inulin was seen. We conclude from these results that (1) exposure to microwaves or

to a warm-air environment resulted in a significant increase in general brain permeation to sucrose but not to inulin, and (2) that the more conservative regression analyses did not result in any specific brain region having a significant increase in permeation, although insignificant trends were noted.

This experiment was designed to correct for the effect of increased core temperature on permeation. The results show clearly that exposure to microwaves at the indicated levels imposed a thermal load; therefore, the results from the warm-air exposure study were used to correct the microwave data for permeation due to the increase in temperature. The variability of these data (see Table 4) caused a large variation in the value of S_i, one of the terms in the correction factor, as shown in Table 5. However, after correcting the inulin data by this procedure the P value changed from .107 to .223 indicating that at least part of the variability in the data can be explained by microwave heating. The microwave-sucrose data were also corrected by this procedure. Before the correction was made the data showed a significant increase in sucrose permeation with respect to microwaves ($P = .026$), while after the correction the permeation was no longer significant. This indicates that (1) a correction for the influence of core temperature on permeation is valid and that (2) this correction explains part of the trend toward higher permeation in the animals exposed to microwaves.

It has been suggested that the relationship between brain permeation and temperatures has a region of minimal permeation at or near normal body temperature [Justesen, 1980]. If this suggestion is correct, permeation varies in a more complex way with temperature than would be predicted by our linear model. However, the data in Tables 2–4 do not describe a curvilinear function which has a well-defined region of minimal permeation; the results show that K_i, in general, remained constant or increased as rectal temperature increased. Over the span of body temperatures in this study, we believe the correction factors to be conservative values that underestimate the thermalizing effect of the microwave radiation in the corrected data. Future work will determine whether the true relationship between permeation and body temperature is linear as assumed here, or multiphasic. One should note that our conservative estimate of the effect of temperature on brain permeation was sufficient to account for the increase in permeation caused by the microwave exposure. In addition, one should note that a general increase in sucrose permeation was found in rats exposed to warm-air environments of 22, 30, or 40 °C. Under these conditions, the rectal temperature of the anesthesized animals did not exceed 38.4 ± 0.2 °C (Table 2) and the brain temperature in each of the eight regions does not exceed 38.5 °C (unpublished data).

In this experiment two radioactive saccharides, sucrose (MW 342) and inulin (MW 5000), were used to compare permeation by relatively large and small molecules and to gain some insight into the mechanism by which microwaves and conventional heating affect brain permeation. For instance, if the permeation by both tracers increased equally and large increases were seen, one might conclude that the tight junctions between the endothelial cells had been opened. But if permeation of both tracers increased only slightly, then one could suggest that the pinocytotic activity had increased. Another possibility would be for permeation of the smaller molecule to increase but not the larger; this would indicate that there was an increased leakiness through the membrane. We did find a significant increase in permeation of the smaller molecule, sucrose, but no change was found with inulin. Therefore, the results indicate that the increased permeation was dependent upon molecular size and was due to an increase in the leakiness through the capillary endothelial cell membranes.

Since 1975, three sets of investigators who have used different techniques have reported that microwave radiation at power densities at or below 10 mW/cm^2 have caused

changes in the blood–brain barrier: Frey et al [1975] found that uptake of a fluorescent dye into the brains of animals exposed for 30 min to pulsed fields (1.2 GHz; average power density = 0.2 mW/cm^2) was greater than that from CW exposure (1.2 GHz; 2.4 mW/cm^2), and that both were greater than control values. Under light and electron microscopy, Albert [1977] observed alterations in the permeation of the blood–brain barrier to horseradish peroxidase (MW = 40,000) in Chinese hamsters exposed to 2450-MHz (CW) fields at 10 mW/cm^2. In a later study, Albert [1979b] found that 12 of 22 hamsters and 5 of 8 rats showed increased permeation by horseradish peroxidase immediately after CW irradiation at 2800 MHz (10 mW/cm^2 for 2 h). Oscar and Hawkins [1977] used Oldendorf's technique to measure the uptake of mannitol (MW = 182), inulin (MW ≅ 5000), and dextran (MW = 60,000–75,000) in the brains of rats exposed to either CW or pulsed radiation (1.3 GHz). This technique measured the change in permeation during one capillary pass (~15 s) of arterial blood after irradiation had ceased. Increased mannitol and inulin uptakes were found in the hypothalamus, cerebellum, and medulla, but only the cerebellum had an increase in dextran uptake; 24 h after exposure, the uptake of mannitol in the medulla was significantly increased. It is of interest that, mannitol uptake varied with power density for the different modulation conditions, eg, maximal uptake occurred at about 1 mW/cm^2 for CW exposure between 0.3 and 3.0 mW/cm^2.

Attempts to replicate parts of the above studies or to use the same techniques at different frequencies have failed to confirm the effects of microwave radiation on the blood–brain barrier. Merritt et al [1978] could not replicate the changes reported either in the fluorescent experiment of Frey et al [1975] at 1.2 GHz (pulsed) or in the mannitol uptake study of Oscar and Hawkins [1977] at 1.3 GHz (pulsed). Preston et al [1979] used Oldendorf's technique to determine if a 30-min exposure to 2450-MHz (CW) radiation (0.1–3.0 mW/cm^2) increased the uptake of mannitol; no increase was reported. More recently, Lin and Lin [1980] reported no permeation of Evans blue and sodium fluorescein dyes into the brains of anesthesized rats exposed to microwaves (2450 MHz, pulsed) under conditions that caused the brain temperature to increase ~4 °C to about 41 °C.

It is apparent from the above studies that substantiation of a low-level microwave effect on the blood–brain barrier has not been firmly established. Additional research is needed to determine if the reported effects are artifacts or, if the differences in irradiation parameters, biological methods, or the thermal influence of microwaves can explain the discrepancies in the experimental results.

These three points were addressed in the present study in the following ways. First, microwave parameters used in this study were very similar to those used by Albert [1977] ie, 2450 MHz, 10 mW/cm^2, CW. Second, the experimental procedure used in this study is from two to three orders of magnitude more sensitive than the Oldendorf technique [Blasberg, 1979]. Furthermore, the histological techniques used by Frey et al [1975], Albert [1977, 1979b], and Merritt et al [1978] are sensitive but they are semiquantitative at best. This study also addressed the transient nature of the effect reported by Oscar and Hawkins [1977] and Albert [1979b] by monitoring barrier permeation during exposure. Third, an attempt was made to correct the changes in permeation during microwave radiation for the change in permeation associated with whole-body heating. We feel the results of this study should aid in the clarification of the apparent discrepancies.

In their recent report, Preston and Préfontaine [1980] used an experimental protocol similar to the one described here. That is, brain uptake of sucrose was measured in rats during a 25-min exposure to 2450-MHz CW radiation. Their study differed in that they

exposed their rats in the near field at SAR of 0.08, 0.30, or 1.6 W/kg while in our experiment rats were exposed in the far field at SARs of 2, 4, or 6 W/kg. Preston and Préfontaine [1980] reported no effect of 2450-MHz microwave radiation on the blood–brain barrier. At higher SAR, we did find a general increase in brain permeation to sucrose, however, the change in permeation was found to be due to the thermalizing effect of the microwave exposure.

DISCLAIMER

This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation of use.

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